TRANSFUSION SUPPORT IN LEUKEMIC CHILDREN

Dissertation submitted to the

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CONTENTS

PAGE NO.

1. INTRODUCTION

The incidence of different types of leukemia varies with age through out the world.[1] In India, Parkienstal et al reported that leukemias are the most common cancer affecting the children accounting for 25 – 35% of malignancies.[2] The majority of them were ALL and most were in age groups 0 – 3 yrs, 4 – 6 yrs and 7 – 9 yrs. ALL is most frequent in South India[3.] In Chennai, leukemia was reported as the highest among the first five childhood cancers.[1]

Acute leukemia is a malignancy arising due to the clonal proliferation of abnormal hematopoietic cells leading to disruption of normal marrow function resulting in increased number of blast cells > 20% (Normally blast cells <5%).[4]

Patients with acute leukemia undergoing induction chemotherapy have prolonged thrombocytopenia, because of the cytotoxic therapies they receive and also of their underlying disorder. Bleeding is a frequent complication in leukemic children even after prophylactic or therapeutic platelet transfusion.[5]

Valleri et al suggested that transfusion of RBC to anemic thrombocytopenic patients would be effective before transfusing viable platelets. Maintenance of hemoglobin concentration of a patient with thrombocytopenia at higher levels may contribute to improve hemostasis.[6]

In tertiary care centres, platelet transfusions are widely used for the management of bleeding in thrombocytopenic leukemic patients.[7] Clinicians faced with an abnormal laboratory value may use transfusion therapy to correct the value rather than to achieve a clinical result.[8] Prophylactic transfusion may or may not improve platelet survival when compared to transfusion in response to active bleeding.[5]

A reliable platelet count and appropriate clinical evaluation of the leukemic patients showed that a significantly lower threshold is needed more for therapeutic transfusion than for prophylactic transfusion.[9]

For the past 30 years platelet transfusion have been used in the treatment of thrombocytopenia caused by decreased production, inadequate function or increased destruction of platelets. The number of platelet transfusion has increased in leukemic patients due to

aggressive chemotherapies producing more acute and prolonged thrombocytopenia.[10] So the present study was undertaken to find the

(i) need for transfusion in leukemic children and

(ii) to evaluate the effectiveness of RBC and platelet transfusion for them.

2. AIMS AND OBJECTIVES

(i) To study the need for transfusion in leukemic children.

(ii) To identify the blood component most commonly used for transfusion.

(iii) To find the pre and post Hemoglobin estimation.

(iv) To find the pre and post platelet count increment.

3. REVIEW OF LITERATURE

3.1 Methods of component preparation

Platelet rich plasma (PRP) can be separated from whole blood by a soft centrifugation. The processing of blood and PRP are done at room temperature (22°C) and holding the sedimented platelets stationary for 30 – 60 minutes before resuspending them as platelet concentrates (PC).[11] Platelet rich plasma was the first method used to prepare PC[12]. Platelet concentrates from Whole blood are often referred to as “random donor platelets”. Platelet concentrates are prepared by centrifugation of standard amounts of whole blood.[13] Preparation of platelet concentrates are done from anti coagulated whole blood by the “platelet rich plasma” and or “buffy coat techniques”.[11]

There are two methods for doing platelet preparation

(i) Platelet rich plasma (PRP) method.

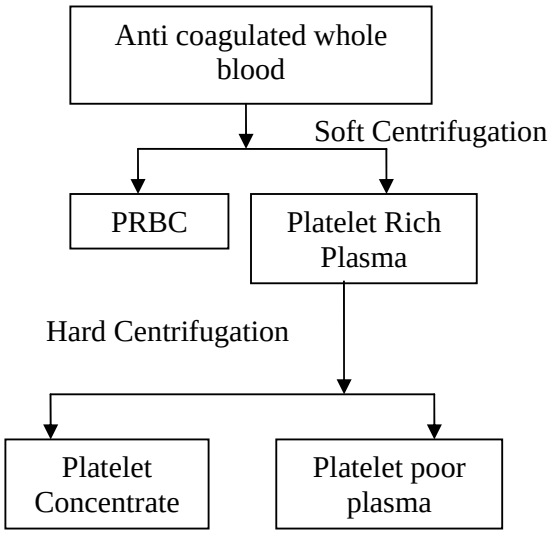
(ii) Buffy coat (BC) Method.

In the PRP method, an initial Low G force (soft) spin produces PRP, which is then separated from the packed red cells. PRP is then centrifuged at a higher G force (hard) spin and the supernatant platelet poor plasma is removed. The residual PC contain approximately 0.5 to

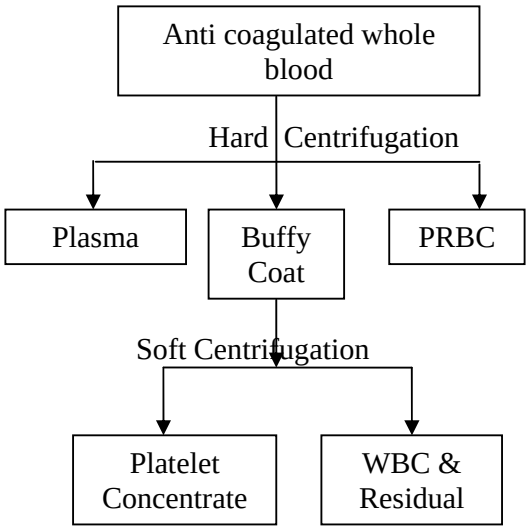
0.75 x10^{11} platelets / unit or approximately 60to 75% of the platelets from the original unit of blood.[13] One draw back is that this method results in 10^{8} WBC or approximately 50% or more Leukocyte contamination occurs during seperation from the original unit of Whole Blood.[13]

In the second method i.e. Buffy coat method, anticoagulated whole blood is subjected to hard spin, resulting in separation of platelet poor plasma (supernatant), buffy coat (intermediate) and PRBC at the bottom. Buffy coat is separated and subjected to soft spin leading to separation of PRP and WBC with residuals.

Platelet Rich Plasma Preparation



Buffy coat preparation



3.2 Storage

Platelets are stored at 20˚C to 24˚C using continuous gentle horizontal agitation in storage bags specifically designed to permit Oxygen and Carbon dioxide exchange to optimize platelet quality.[13] This combination of storage bag, constant agitation preservative solution, temperature and the use of approximately 50 ml of plasma permit satisfactory preservation of platelets up to 5 days.

3.3 Transfusion

Platelet storage also has an impact on platelet transfusion efficacy. Transfusion with platelets stored from day 0 to day 5 were

used[14]. All the platelets used were ABO group identical between Donor and the recipient. A single unit transfusion was considered as one transfusion episode and is defined as either RBC unit or unit of "whole blood derived platelet concentrate".[14]

3.4 Hematology Analyzer

In blood banks hematology analyzers, are used to count cells in blood products. These blood products are derived from centrifuged whole blood.[15] Platelet counting in platelet concentrates should be validated separately for platelet collected from whole blood because most of the analyzers are designed to count platelets in the presence of RBC. Platelet counting in the absence of RBC might result in deviating from correct platelet counts. The samples were taken in a EDTA tube. Significantly higher platelet count in the EDTA tube was observed by the cell analyzer than in the dry tube.[15]

3.5 Role of RBCs in bleeding

Intensive therapies which produce severe and sustained thrombocytopenia are used routinely in patients with hematological malignancies.[13] Guidelines for transfusion exist, but variability in their application, particularly in children, remains as a big concern.[1] Non

surgical blood loss refers to generalized, systemic bleeding that is not corrected by surgical interventions. A correlation exists between anemia and prolonged bleeding time. When the anemia is corrected, the bleeding time is reduced. Platelet function is known to be a major determinant of bleeding time and anemia produces a reversible platelet dysfunction.[6]

RBCs show beneficial effect on platelet function by dispersing platelets from the centre of the blood vessel towards the vessel wall, RBCs concentrate platelets near the endothelial cells of the blood vessel, where they are balanced to respond to injury.[6] Haemostatic effect of RBC is to scavenge endothelial cell and nitric oxide, a vasodilating agent that inhibits platelet function. In addition, Endothelin, a potent vasoconstrictor substance may be released from endothelial cells. RBCs, like platelets at the bleeding site may provide arachidonic acid and adenosine di-phosphate to stimulate the platelets to release thromboxane, another potent vasoconstrictor substance and a platelet aggregating substance.[6] At the bleeding site, the platelets and RBC's are activated and phosphatidyl serine is exposed on both the platelets and RBC's. Factor Va and Factor Xa activates prothrombinase to produce thrombin, which accumulates on the platelets and RBCs.[6]

A Hematocrit level of 35 volume percent at the site of bleeding minimizes shear stress and reduces nitric oxide produced by endothelial cells.[16] Therefore trigger for prophylactic platelet transfusion should consider both the Hematocrit and the platelet count. If WBC depleted platelets are used, it will modify the development of allo immunisation in leukemia.[17]

Pooled platelet products and single donor apheresis platelet concentrates are considered to be equally effective and safe.[18] A standard dose of 0.5×10^{11} platelets per 10 kg for platelet transfusion is generally accepted.[18] Children who need blood transfusion receive a calculated volume of blood depending on their weight, instead of whole number of units.[19] Acute Leukemic children during therapy receive on an average 80 – 110 units of platelets and 20 – 40 units of red cells.[20] There should be documentation on the patients chart indicating whether the patient had a chance to discuss about the need for blood transfusion and its procedure. The documentation has considerable importance in terms of recognizing a delayed transfusion reaction or a transfusion transmitted diseases.[15] Clinical Judgment is the principal factor in determining whether a RBC transfusion is indicated. The benefits of the hematocrit range of 30 to 35 percent includes improved Oxygen delivery

and enhanced hemostasis.[6] Haemoglobin level increase is always associated with the transfusion of red cells in anemic cancer patients.

3.6 Clinical features

In leukemia, the blast cells replace normal hematopoietic cells in the marrow resulting in Anemia, thrombocytopenia and neutropenia. Most of the clinical symptoms are due to anemia, neutropenia and thrombocytopenia.[21]

The most common presenting clinical features of leukemia are :

1. Fatigue and pallor
2. Respiratory infection – Brochopnemonia
3. Bone pain – sternal tenderness
4. Lymphadenopathy
5. Hepatosplenomegaly
6. CNS involvement and
7. Petechial hemorrhages and mucosal bleeds

3.7 Hematological findings in Leukemia

The presence of blast cells in Blood and Bone marrow with reduction of normal hematopoietic cells is the hall mark of leukemia.

Peripheral smear reveals blast cells which vary from as few as 1% to as high as 98 – 99% .Blast cells are either lymphoblasts in ALL or myeloblasts in AML. Lymphoblast may be of L1 L2 or L3 morphology and demonstrate block positivity with Periodic Acid Schiff stain. Myeloblasts are positive for Sudan Black and Myeloperoxidase B stains.

3.8 Bleeding in Leukemic Children

In leukemic children, skin bleeding (Petechiae) is a sufficient clinical sign to transfuse platelets.[22] The other common clinical indicators for therapeutic platelet transfusion are gastrointestinal, genitourinary (Hematuria) and retinal hemorrhages. Significant bleeding included all bleeding except petechiae formation in dependant areas, ecchymoses not larger than 1 cm in diameter and/or more than 5 in number or oozing of blood from the periodontal groove.[23]

3.9 Categorization of bleeding events

The bleeding event is categorized as no bleeding, non clinically significant bleeding and clinically significant bleeding and the corresponding WHO grade and symptoms are mentioned below.[9,6]

Table 1 Categorization of bleeding events

Bleeding	Category Corresponding WHO Grade	Description of bleeding Signs / Symptoms
No bleeding	0	No bleeding
Non clinically significant Bleeding	1	1. Petechiae 2. Purpura 3. Mucocutaneous hemorrhage 4. Mild vaginal spotting 5. Mildhemorrhoidal spotting 6. Oropharyngeal bleeding 7. Epistaxis not requiring Packing or intervention 8. Retinal hemorrhage without visual impairment 9. Hematomas not requiring red cell transfusion
Clinically significant bleeding	2, 3 or 4	1. Malena 2. Hematomas 3. Hemoptysis whether or not blood Transfusions where required (WHO> 2) 4. Hematuria 5. Hematochezia 6. Abnormal vaginal bleeding 7. Epistaxis requiring package or intervention 8. Bleeding from invasive sites 9. Musculoskeletal bleeding 10. Hematoma (soft tissue bleeding) 11. Body cavity fluids reported as grossly bloody

3.10 Role of platelets in bleeding

The life span of platelets is dependent on a patient's platelet count. Although a platelet survives approximately nine days in a normal individual, platelets have a reduced survival in thrombocytopenic patients. The residual mean life span corresponds to the maximal expected life span of a donated platelet product after infusion into the patient. The explanation offered for these observed differences is that the platelets are removed from the circulation by two mechanisms. First is simply platelet senescence which accounts for majority of platelet loss in normal individual and the second is a constant loss due to the routine maintenance of vascular integrity (an endothelial supportive role). The number of platelets required for this endothelial supportive role has been estimated to be $7.1 \times 10^3/\mu l$/day. It is thought that in thrombocytopenic patients the constant loss for haemostatic maintenance represents a more significant fraction of the circulating platelets, thus reducing platelet survival[9].Certain clinical factors also influence the in vivo survival of activated platelets which may be the major cause of refractoriness to platelet transfusion.[10,24]

3.11 Prophylactic platelet transfusion

Prophylactic platelet transfusion is an important part of supportive care measures in modern treatment. The aim of prophylactic transfusion is the prevention of major as well as minor bleedings during the time of disease or therapy induced thrombocytopenia.[18] Hospitals have tried to reduce platelet use and the cost of platelet transfusion by transfusing platelets at lower platelet counts more frequently. But it is not optimal for cancer patients receiving intensive chemotherapy who require multiple platelet transfusion.[10] There are studies determining the optimal transfusion trigger for prophylactic platelet transfusions in patients who have chemotherapy induced thrombocytopenia. Most of the centers now use a trigger of $10x10^9$/L to transfuse platelet products.[25]

More than 70 percent of hospitals reported transfusing platelets primarily for prophylaxis. 60 percent of the hospitals set the threshold platelet count for prophylactic platelet transfusion at 20,000/µl. A platelet count of 50,000/µl was most frequently required for performance of a minor invasive procedure. Finally the authors concluded that there is a considerable variation in platelet transfusion practice. For pediatric patients, in particular, the dose of platelets transfused was individualized on the basis of the weight of the child.

Among 20% of institutions surveyed reported that, among clinicians, the most common reason for platelet transfusions was for hemostasis in response to active bleeding.[20]

Recent studies suggest that the threshold for prophylactic platelet transfusion may be safely lowered to $10x10^9$ / L from previous standards of $20x10^9$/ L Earlier studies of spontaneous bleeding demonstrated that bleeding risk increased dramatically only at platelet counts below $5x10^9$ litre.[26] In current practice, most platelet transfusion is given for prophylaxis. one random unit/10 kg for pediatric patients is the usual dose. Platelet survival varies with platelet count because of consumption of a fixed number of platelets each day to maintain vascular integrity. If prophylactic transfusion for severe thrombocytopenia are intended to maintain vascular integrity, then the total platelet usage may be determined by that fixed consumption, rather than by senescence of excess transfused platelets.[26]

73% of the platelet transfusions that were given to the patients occurred with pre transfusion counts of more than $10x10^9$/L which is the pre transfusion count trigger for prophylactic platelet transfusion that is now generally accepted in the literature. This mean next day increment for all transfusion, 10.2 x 10^9 / L is much lower than the $30x10^9$/L that

is often cited as the rule of thumb for the expected increase in uncomplicated patients.[5]

Clinical trials have shown that one hour post transfusion count is a good measure of response to transfusion whereas the 24 hour count can be used to monitor platelets survival. Astler and Jandl showed that the recovery of platelets when labeled and re-infused into an autologous normal donor is not 100 percent but rather 52 percent. The decreased recovery is attributed to pooling of normal platelets in the spleen. This pooling is not reversible as platelets go back and forth between the spleen and the general circulation. On an average, one platelet is in the spleen for two in the circulation.[27] A fixed number of platelets is required for hemostasis each day. As thrombocytopenia worsens, the percentage of platelets used each day increases. So the choice of 50 percent for mean cell life (MCL) was based on physiological and medical considerations.[27]

Some institutions did not use any specific platelet count for prophylactic transfusion, because the patient's clinical condition played an important role in the decision to transfuse. It is inappropriate to automatically administer the platelet transfusion at a given platelet count

and that the number of platelet transfusion administered to many patients can be decreased with proper clinical evaluation.[20]

3.12 Group matched platelets

It has been an accepted practice for platelets to be transfused out of ABO group as a second line therapy when ABO matched platelets are unavailable. The mismatch is tolerated in most cases owing to the assumption that the antibodies and volume of incompatible plasma transfused will be diluted in the total blood volume of the recipient. This dilution prevents passively acquired ABO antibodies from causing a clinically significant problems.[27] Intravascular hemolysis secondary to minor ABO mismatch is defined as donor plasma containing isohemagglutunin to antigens on recipient RBC causing severe morbidity and mortality. This is especially true for those transfusions of group O plasma or platelets to group A, B or AB recipients.

Passively acquired antibodies are detectable in recipients only when the donor antibody titres are high. In these cases the transfusion reactions ensue when the plasma contains a high titre antibody, when large volume of plasma are transfused or when small children and infants received transfusion. Most of the hospital services do not

routinely measure isohemagglutunin titres (IgM or IgG) before transfusing ABO mismatched plasma, although some recommend this. A policy was instituted to test anti A, anti B, anti AB titres of all group O Single Donor Platelets before transfusion to group A patients. If IgM> 1: 64 and or IgG is > 1:256, the platelet product is not transfused out of group.[27]

3.13 Leukemia and Alloimmunisation

Alloimmunisation represents the major complication of platelet transfusion therapy for patients with acute leukemia.[28] Platelet refractoriness defined as two or more consecutive uncorrected post transfusion platelet count increments of less than 4,000 per ml. Patients who become alloimmunized following induction will continue to require HLA matched platelets. However, patients who do not become immunized during induction can easily and repeatedly be transfused with random donor platelets and can therefore be given subsequent prophylactic transfusions liberally without concern for the induction of alloimmunisation.[28]

3.14 Corrected count increment (CCI)

The corrected count increment has been used as a surrogate measure for bleeding in platelet dose studies and also for platelet transfusion triggers studies.

$$CCI = \frac{\text{Platelet count increment (Post count – Pre count) X BSA}}{\text{No. Of Platelets in the transfused products (X } 10^{11}\text{)}}$$

CCI may not be a valid and reproducible surrogate marker as it depends upon (a) BSA – Calculation of BSA is variable as per the formula used. (b) Total No. of Platelets in the Platelet product transfused.[25]

Hematology Laboratory using electronic counter were not designed to accurately count cells in platelets rich plasma. The potential sources of variation in results includes

1. Method of taking the sample
2. Type of test tube that the sample is placed (plastic or glass)
3. Duration of time that the sample sits before being counted
4. The accuracy of making the dilution
5. The instrument on which the counting is being performed

There are many studies which showed that there is significant variability in counting depending on the instrument that is being used. BEST study demonstrated the variability in platelet counts performed on samples of platelet rich plasma in eight different laboratories in five different countries. 20 – 60% of patients requiring regular platelet transfusions failed to achieve adequate post transfusion increments and are considered to be refractory to platelet transfusion.[29] The factors influencing CCI are 1. Splenectomy, 2. Bone marrow transplantation, 3. Concurrent IV administration of Amphotericin B., 4. Palpable Spleen, 5. Temperature.[28] Amphotericin B also binds to the sterol moiety of membrane to produce increased permeability and lysis of red cells.[28]

3.15 Guidelines for platelet transfusion in infants

The goal of most platelet transfusion is to raise the blood platelet count to greater than 50 X 10^9/L. Transfusion of 10ml/Kg of platelet concentrate provides approximately 10X10^9 platelets. Considering the blood volume of an infant is 70ml / kg body weight and the recovery of transfused platelets to be 50%, a platelet dose of 10ml / kg will increase the immediate post transfusion platelet count by 100 -150 X 10^9/L.[30]

3.16 Factors that affect the response to platelet transfusion[10]

Table 2 Factors that affect the response to platelet transfusion[10]

Clinical	Patient	Blood bank
Fever	Alloimmunization	ABO mismatch
Sepsis	Disease	Storage time
Splenomegaly	Drugs	
Bleeding	BMT recipient	
DIC		
Platelet count		

4. MATERIALS AND METHODS

4.1 Study Design and Place

- An Observational Study conducted in the Department of hematology, Institute of Child Health during the period 2007 – 2008. The study population included children diagnosed as acute leukemia in the age group 1 – 12 years.

4.2 Inclusion Criteria

- All acute leukemic Children, 1 – 12, years both male and female.
- Children diagnosed as acute lymphoblastic leukemia and acute myeloid luekemia.
- All acute leukemic Children requiring transfusion support.
- Both treated and follow up children.

4.3 Exclusion Criteria

- Leukemic children below 1 year and above 12 years.
- Lymphoma evolving to leukemic phase.
- Juvenile myelomonocytic leukemia.
- Not willing to participate in this study.

4.4 Variables Studied

- Age, Sex, Weight
- Hemoglobin Level
- Platelet Count
- Hematocrit

4.5 Sample Size

Sample Size is calculated by the formula $N = Z^2 P (1-P) / d2$

Where N = Sample Size

Z = Z statistic for a level of confidence.

P = expected prevalence or proportion

d = Precision

Z statistic (z): CI-95%, Z value is 1.96

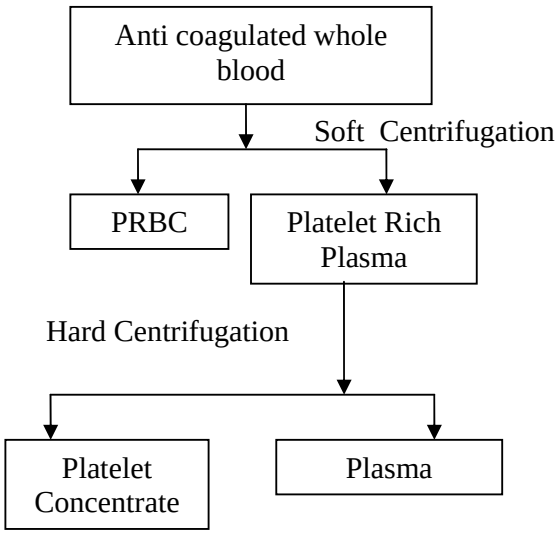
N = (1.96)2 x 0.1 (1-0.1) / (0.05)2 = 135

(This is for 10% Prevalence & for 20 - 50% Prevalence it is 245)

So the total sample taken for the study is 250 and in this the transfusion of each unit is taken as one sampling unit.

4.6 Components prepared

The components used in this study were platelets and packed red cells and were prepared as follows:



The red cells were stored at 2-6°C (shelf life 35 days) and the platelets were stored at 20-24°C (shelf life 5 days) under constant gentle agitation.

The platelets were neither leukoreduced nor irradiated. No apheresis platelets were used.

The transfusion was given depending upon the clinical sign of bleeding and the hemoglobin estimation. The following data have been documented in this study for analysis.

The hematology analyser used in this study was Sysmex4000 working on the principle of Impedance .The pre and post transfusion hemoglobin and platelet count were estimated 24 hours after the transfusion.

Hemoglobin content of the RBC and platelets were estimated as a quality measure once in a month randomly. The hematology analyser used was calibrated once in three months using known samples.

4.7 Variables

Before transfusion the following data were documented and the variables used in the study are

- Pre Hemoglobin count
- Pre platelet count
- No of units of RBC and platelets transfused
- Post hemoglobin count after 24 hrs of transfusion
- Post platelet count after 24 hrs of transfusion
- Clinical signs of bleeding and platelet count.

5. RESULTS

Two hundred and fifty episodes of transfusion given to 30 leukuemic children were analysed . The data is analysed as follows:

5.1 Demographic details

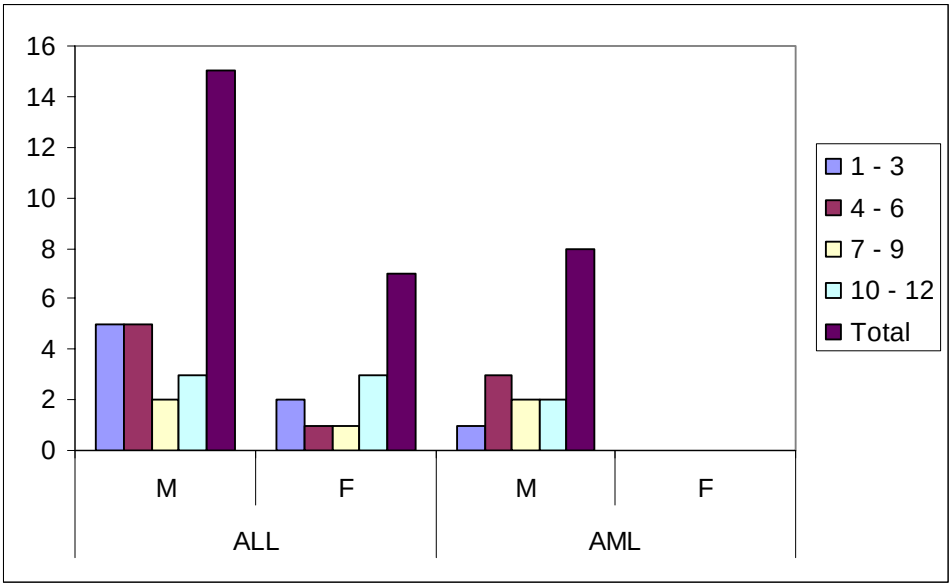
5.2 Clinical features

5.3 Analysis of transfusion

5.1 (a) Demographic details

Table 3 Age & Sex distribution

Age (in years)	ALL		AML	
	M	F	M	F
1-3 years	5	2	1	0
4-6	5	1	3	0
7-9	2	1	2	0
10-12	3	3	2	0
Total	15	7	8	0

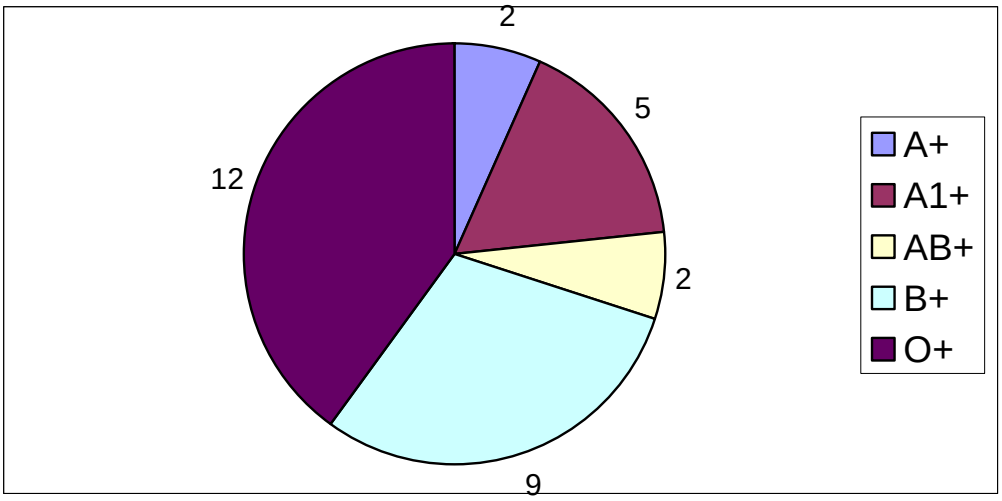


From the above details, 76.67% were males and 23.33 were females. In leukemia 73.33% were acute lymphoblastic leukemia and 26.67 were acute myeloid leukemia.

5.1 (b) Blood group distributions

Table 4 Blood group distribution

Blood Group	Number of children
A+	2
A1+	5
AB+	2
B+	9
O+	12
Total	30



Here 40% of the leukemic children belonged to O group which is similar to the general distribution. It is unusual that none of them were Rh negative whereas in the general population it is 15%.

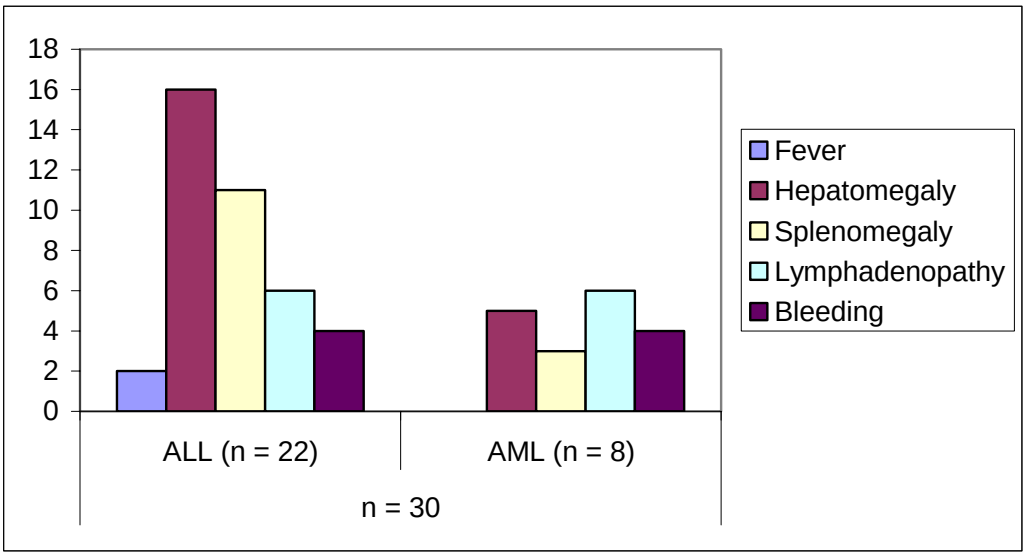
5.2 Clinical Features

The Leukemic children presented with the following major clinical features

1. Fever
2. Hepatomegaly
3. Splenomegaly
4. Lymphadenopathy
5. Anemia
6. Bleeding

Table 5 Clinical features

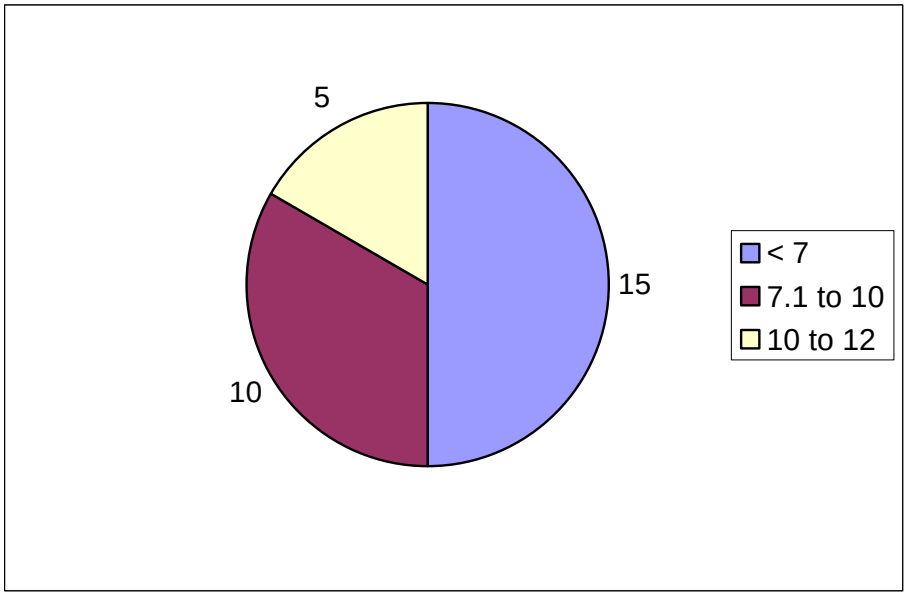
Clinical Features	n=30	
	ALL n=22	AML n=8
Fever	2(9.1%)	0
Hepatomegaly	16(72.7%)	5(62.5%)
Splenomegaly	11(50%)	3(37.5%)
Lymphadenopathy	6(27.8%)	6(75%)
Bleeding	4(18.2%)	4(50%)



Hepatomegaly and Splenomegaly were common among the clinical features and 22% of the patients presented with bleeding.

Table 6 Presence of Anemia

Hb% in grams	No. Of Children
< 7	15
7.1 to 10	10
10 to 12	5

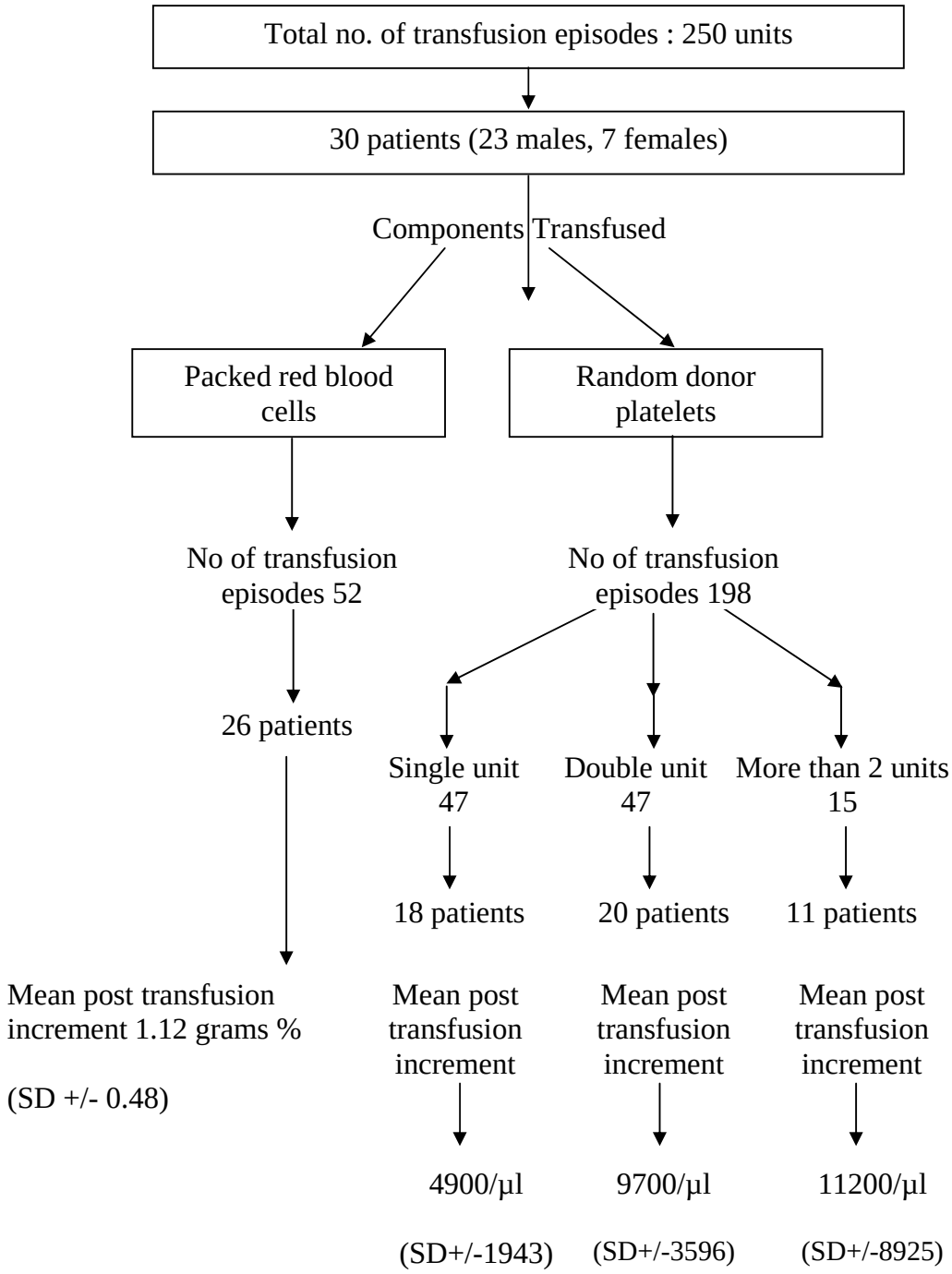


At the time of presentation 5 of the children had hemoglobin > 10gms %. 15 children had hemoglobin < 7gms % and among them 2 presented with bleeding and 10 children had hemoglobin between 7.1 to 10gms % and among them 4 presented with bleeding. Cause and effect of anemia with bleeding could not be ascertained from their presentation.

5.3 Analysis of Transfusions

30 Leukemic children received 250 transfusions. 52 of them were packed red blood cells. 198 of them were platelets. Of this 47 platelet transfusions were single unit, 33 received double units and 16 received more than 3 units at a time. Pre Hemoglobin and post Hemoglobin estimation and the pre platelet and post platelet count were done after 24 hours of transfusion.

Algorithm of Transfusion episodes



5.3 (a) Clinical Factors Influencing Platelet Count

Table 7 Bleeding Vs Platelet count

Bleeding	Platelet Count < 5000 / µl	Platelet Count > 5000 / µl
Present	4	4
Absent	1	21

Odds ratio = 21($p < 0.05$)* Bleeding is definitely associated with thrombocytopenia.

Table 8 Splenomegaly Vs Platelet Count

Splenomegaly	Platelet Count < 5000 / µl	Platelet Count > 5000 / µl
Present	2	12
Absent	1	15

Odds ratio = 2.5 ($p<0.05$)* Presence of Splenomegaly is also connected with low platelet count.

Table 9 Fever Vs Platelet Count

Fever	Platelet Count < 5000 / µl	Platelet Count > 5000 / µl
Present	1	1
Absent	4	24

Odds ratio = 6 ($p<0.05$)* Presence of fever also contributes to thrombocytopenia.

Table 10 Distribution of platelet count in children with bleeding

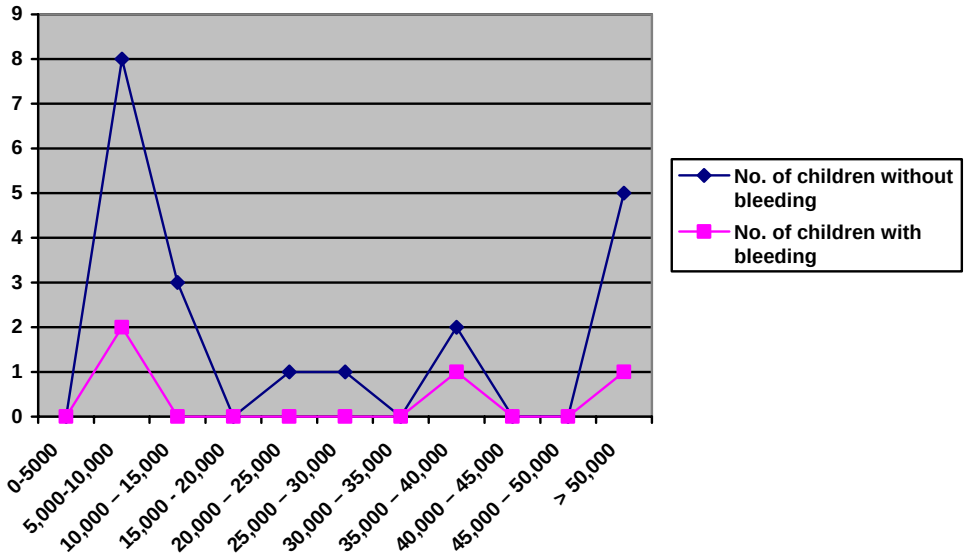
Platelet Count	No. of children with bleeding
< 5000/µl	4
> 5000/µl	4

8 children presented with bleeding and 50 % had platelet count < 5000/µl and 50 % had platelet count > 5000/µl.

Table 11 Distribution of platelet count in children without bleeding

Platelet Count in μl	**No. of children without bleeding**
0-5000	0
5,000-10,000	8
10,000 – 15,000	3
15,000 - 20,000	0
20,000 – 25,000	1
25,000 – 30,000	1
30,000 – 35,000	0
35,000 – 40,000	2
40,000 – 45,000	0
45,000 – 50,000	0
> 50,000	5

Here 20 children presented without bleeding as shown in the above table.



Statistically there is no correlation with the platelet count between the number of children with and without bleeding

5.3 (b) Analysis of RBC transfusions

52 packed red cell transfusions were given. In order to find the post hemoglobin level rise, the following analysis was done and their analysis is as follows:

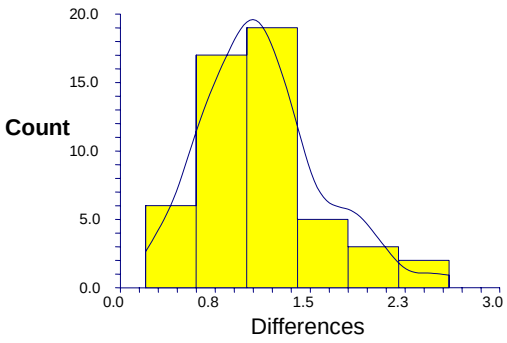
Table 12 Analysis of RBC transfusions

Paired samples t-test n = 52

	Pre transfusion	Post transfusion	P
Sample size	52	52	P < 0.0001
Mean of the Hb level in gms %	6.7346	7.8558	
95% CI for the mean	6.2163 to 7.2529	7.3471 to 8.3645	
Variance	3.4662	3.3386	
Mean difference	1.1212		
Test statistic t	16.846		
Degrees of Freedom (DF)	51		

In the above analysis it is found that there is a mean difference of 1.1212 grams % and for 95% CI for the mean p < 0.0001, the post transfusion rise in Hb is significant.

Histogram of differences



5.3 (c)Analysis of Platelet Transfusions

Out of 198 platelet transfusion analysis about the Pre & Post platelet count were done for single unit, double unit & for 3 & 4 units separately as follows:

Single unit platelets transfusion	=	47
Double unit platelets transfusion	=	51
More than 2 units platelets transfusion	=	15

5.3 (d) Single Unit Platelet Transfusion

After giving 47 single unit platelet transfusion to 18 leukemic children the following analysis were done to find a rise in post platelet count increment.

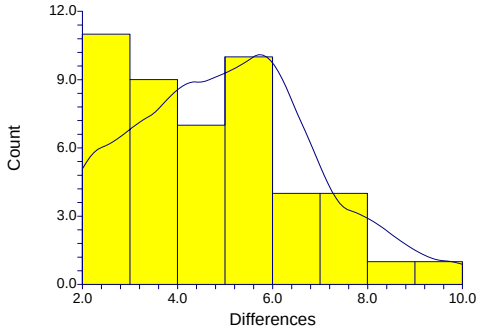
Table 13 Analysis of single unit platelet transfusion

Paired samples t-test n = 47

	Pre transfusion	Post transfusion	P
Sample size	47	47	P < 0.0001
Mean of the platelet count in 10^3 µl/l	39.3745	44.3553	
95% CI for the mean	30.4899 to 48.2590	35.3566 to 53.3541	
Variance	915.6493	939.3299	
Mean difference	4.9809		
Test statistic t	17.572		
Degrees of Freedom (DF)	46		

In the above analysis it is found that there is a mean difference of $4.9809X10^3$ µl / l from the pre and post platelet single unit transfusion. For 95% CI for the mean, the P < 0.0001 and so the rise in post transfusion platelet count is significant.

Histogram Of Difference



5.3 (e) Double units platelets transfusion

51 double units were given and the following analysis done to find out the post platelet count increment

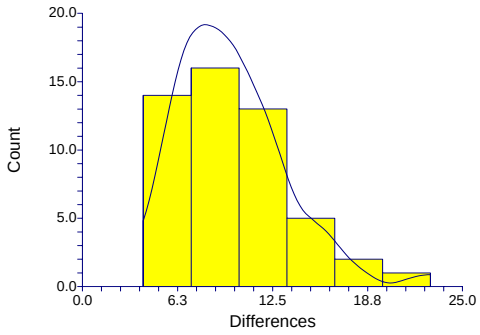
Table 14 Analysis of Double unit's platelets transfusion

Paired sample t-test n = 51

	Pre transfusion	**Post transfusion**	**P**
Sample size	51	51	P < 0.0001
Mean of the platelet count in 10^3 µl/l	35.5843	45.3784	
95% CI for the mean	27.4889 to 43.6798	36.9598 to 53.7971	
Variance	828.4809	895.9477	
Mean difference	9.7941		
Test statistic t	19.448		
Degrees of Freedom (DF)	50		

In the above analysis it is found that there is a mean difference of $9.7941X10^3$ µl/l platelets between the pre and post platelets double units transfusion. For 95 % CI of the mean, P < 0.0001 and so the rise in platelet count is significant

Histogram Of Differences



5.3 (f) Three & Four unit platelet Transfusion

Here the analyses were done for patients receiving more than 2 units of platelets. It was found that 11 of them received 3 units and four of them received 4 units at a time respectively. So these 15 transfusions were analyzed together.

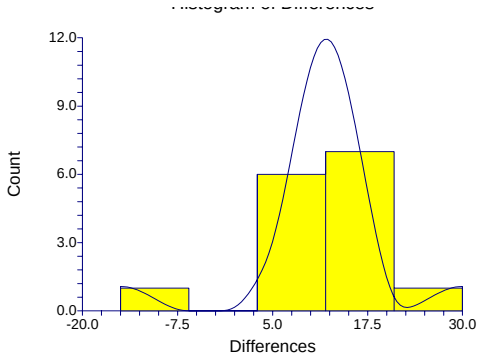
Table 15 Analysis of Three & Four unit platelet Transfusion

Paired sample t-tests n=15

	Pre transfusion	Post transfusion	P
Sample size	15	15	P = 0.0002
Mean of the platelet count in 10^3 µl/l	27.7133	38.9600	
95% CI for the mean	16.9953 to 38.4314	27.0850 to 50.8350	
Variance	374.5898	459.8240	
Mean difference	11.2467		
Test statistic t	4.880		
Degrees of Freedom (DF)	14		

In the above analysis it is found that there was a mean difference 11.2467X10^3 µl/l Platelets between the pre and post platelets transfusion from those who received more than 2 units. For 95% CI for the mean, P < 0.00001 and the rise in platelet count is significant.

Histogram Of Differences



6. DISCUSSION

Hemato-oncology services require many transfusions for a prolonged period. Profound anemia can alter hemostasis and thus should be avoided in patients with thrombocytopenia.[10] Kathryn E. Webert et al hypothesized that maintaining the hemoglobin concentration of a patient with thrombocytopenia at a higher level may contribute to improved homeostasis. In this study, they found that once the patients' hemoglobin level reached the targeted threshold, there were no differences between groups with respect to number of RBC or platelet transfusion.[16] Normal platelet survival is approximately nine days. Hansor SR et al suggested that patients under going induction chemotherapy for leukemia often require platelet transfusion at least every three days. E. Pattern and Visania et al published that acute leukemic patients receive on average 80 – 110 units of platelets and 20 – 40 units of red cells.[20]

In this study, 50 leukemic children were admitted and among them only 30 (60%) children needed transfusion support.

In the present study, out of 250 transfusions given to 30 leukemic children, 55 were packed red cells (mean 1.9 transfusions) and the rest

198 were random donor platelets (mean 2.3). Of 52 packed red cell transfusion episodes given to 22 patients, five of them received more than four units of red cells during the study period. The mean haemoglobin increment for single unit of packed red cell transfusion was 1.12 gms%. 15 children had haemoglobin <7 gms%, 10 of them had haemoglobin level between 7.1 to 10 gms% and 5 of them presented >10 gms%. Of this 15 children, only two presented with bleeding, but among 10 children who had haemoglobin level between 7.1 to 10 gms%, four of them presented with bleeding. Cause and effect of anemia with bleeding could not be ascertain from their presentation. There was no optimal RBC transfusion trigger followed. Out of 198 random donor platelets given to 28 leukemic children, 17 of them received less than 5 units, 8 of them received 5 to 10 units and 3 of them received more than 20 units. The most common component used in the study was platelets and the number of platelets provided per transfusion was not ascertained.

The Number of platelet transfusions has increased more than transfusion of other blood components in leukemic patients primarily because of more aggressive chemotherapies producing acute and prolonged thrombocytopenia. A prophylactic platelet transfusion

approach can prevent bleeding, as opposed to therapeutic approach, in which platelet transfusion is given after a certain degree of hemorrhage has occurred. High et al in 21 patients with acute leukemia found that fever preceded hemorrhage in 10 of the 13 patients who experienced bleeding[23]. In the present study, 2 out of 30 patients (6.3%) had fever and among these two, one had bleeding episode with a platelet count of <5000/μl.

The most controversial aspect of platelet transfusion therapy involves the delineation of the level at which prophylactic platelet transfusion should be administered. Guidelines for the use of prophylactic platelet transfusion are primarily based on clinical experience. In pediatric oncology patients, there are two contrasting points of view. One group tells that the patients should be transfused whenever platelet count falls below 20000/μl, where as the other groups believes that patient should be transfused only when frank bleeding occurs.

Table -16 Summary of Platelet Transfusion Trigger Trials

Study	Year	N-type	Patients
Bayer wl et al	1992	31	Malignancy
Gumur et al	1992	105	Acute Leukemia
Heckman et al	1997	78	Acute Leukemia
Rebulla et al	1997	255	Acute Leukemia
Wandt et al	1998	105	Acute Myeloid Leukemia

Bayer WL et al found that patients with platelet counts less than 6000/µl received prophylactic transfusion, where as those with counts greater than 20000 / µl where transfused only for major bleeding. They concluded that prophylactic level of 5000/µl was safe in the absence of fever or bleeding. Gumer et al, Heckman et al, Rebulla et al have compared the bleeding risk and platelet transfusion needs of groups of thrombocytopenic patients' who received platelets either at the 10000/µl or 20000/µl threshold. They found that there is no difference in hemorrhagic morbidity and mortality rates when the lower platelet transfusion trigger values are used.[13] One major reason for variable practice is based on the need to modify threshold numbers when thrombocytopenia is combined with other complications that increase

the risk of bleeding. In the present study no transfusion trigger was followed. Platelets were transfused to 28 Leukemic children based on their platelet count and bleeding episodes. Out of 198 transfusion episodes given, 8 patients (28.5%) presented with bleeding. In this study 4 of them presented with a platelet count <5000/μl. In this study the clinical signs presented by the leukemic children – bleeding, splenomegaly and fever were analyzed with the platelet count below and above 5000 per μl and the odds ratio calculated and were found to be significant as shown in table 7,8 & 9. The distribution of platelet count children with bleeding and without bleeding were analysed as shown in table 10 & 11. Bleeding was significantly present with platelet count <5000/μl also.

The problems that might have been faced by the physician to decide on an optimal prophylactic threshold could be

(1) Serious hemorrhage was rare at lower platelet numbers.

(2) Presence of minor clinical bleeding.

(3) Accuracy of platelet count when very low platelet numbers found in severely thrombocytopenic patients. In clinical

practice it is difficult to evaluate the efficiency of platelet transfusion due to the fact that

(a) Severe bleeding due to thrombocytopenia alone is rare.

(b) Mortality due to hemorrhage in thrombocytopenia is not common. The bleeding time is not helpful in determining the effectiveness of platelet transfusion. Some times platelet count does not increase to the expected level after platelet transfusion.

Janice P Dutcher et al studied in 114 patients with acute lymphoblastic leukemia who received multiple course of chemotherapy and several platelet transfusions and found that 92% of the patients never become alloimmunized and responded to random donor platelets. Those who remain alloimmunized tended to remain alloimmunized for their entire clinical course. There was no difference in age or sex between groups and prognostic factors predicting alloimmunization . Dutcher in his previous studies also found that there is no dose response relationship between the development of alloimmunization and the number of units of platelets given during induction.[31] In the present study there was platelet increment in all the transfusion episodes and

there was no refractoriness. The mean increment for single unit transfusion (2.6) is 4900/μl and for double unit transfusion (2.6) is 9700/μl and for more than 2 units (1.36) is 11200/μl and the standard deviation is +/- 1943, 3596 and 8925 respectively.

Jeffrey McCullough in 2000 published the data of Williams et al and said that the use of platelet transfusion is associated with increased risk of viral and bacterial infection and alloimmunisation. In the present study of thirty leukemic children all were negative for transfusion transmissible infections. He also found that transfusion reaction occur after 5% to 30% of platelet transfusion and the most common adverse reaction is febrile non hemolytic transfusion reaction which is caused by the patients leukocyte antibodies reacting with leucocytes in the transfused components. In the present study out of 28 children who received platelet transfusion two of them experienced febrile non-hemolytic transfusion reaction.

Table 16 Studies addressing the issue of optimal platelet dose[25]

Study	Year	Patients	N	Dosage of platelets
Noral et al	1998	AML	69	Medium Dose = 4- 6 $x10^{11}$ High Dose = 6 – 8$x10^{11}$ Very High Dose = >8$x10^{11}$
Goodnough et al	2001	Chemotherapy induced thrombocytopenia	120	Medium Dose = 3.4- $x10^{11}$ High Dose = 5.7 $x10^{11}$ Very High Dose = 11$x10^{11}$
Klumpp et al	1993	Human progenitor cell transplant	46	High Dose = 5 $x10^{11}$ Low Dose = 3$x10^{11}$
TinMouth et al	2005	ALL / AML	111	High Dose = 5 WB derived platelets poole Low Dose = 3 WB derived platelets pooled
Sensebe' et al	2005	Acute Leukemia	96	Single Dose = .5$x10^{11}$ Pl / 10kg Double Dose = Twice this amount

In the above studies the transfusion of high dose platelets is associated with the statistically significant increase in transfusion interval and the post transfusion platelet increment. None of the studies adequately addressed the question of optimal dose for prophylactic platelet transfusion.[25]

7. SUMMARY

- All leukemic children included in this study were given transfusion and all of them who were transfused showed significant clinical improvement showing that there is a need for transfusion support in this group.

- Most common blood component used in this study was platelets (79%) and the remaining were red cells (21%).

- There was a mean hemoglobin rise of 1.12g% after transfusion of one unit of red cells.

- The platelet increment were as follows:

  For single unit – 4.98×10^3 / µl

  For double unit – 9.74×10^3 / µl

  For more than 2 units – 11.2×10^3 / µl

  Platelet count increment was present in all transfusion episodes and there was no refractoriness.

  7.14% of leukemic children experienced febrile non-hemolytic transfusion reaction.

All children were negative for transfusion transmissible infections at the end of the study.

- 50% of the children who presented with bleeding had platelet count < 5000 per microlitre.

- Transfusions were based purely on clinical signs and no transfusion trigger was followed.

8. CONCLUSION

There is a definite clinical improvement with transfusion of blood components in leukemic children and also there is no refractoriness to the number of units transfused. The decision to transfuse blood components can be followed based on the clinical signs and symptoms.

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SUB	NAME	IP NO	AGE	SEX	Weight	DIAG	BLOOD GROUP	RBC	PLT	Total	HEP	SPLE	LYMPH	BLEEDING	FEVER	SUBJECT NO & DIAGNOSIS	RBC TRANSFUSION				PLATELET TRANSFUSION			
																	S.NO	PRE HB	NO OF UNITS	POST HB	S.NO	PRE	UNIT	POST
1	GANI	1806/08	3	M	13	ALL	AB +	2	5	7	1	1	2	2	2	1. ALL	1	3.1	1	3.9	3	80x10^3	1	89X10^3
																	2	4.1	1	5.4	4	88x10^3	2	100X10^3
																					5	120x10^3	2	131X10^3
2	HARI ROSHAN	436/08	2	M	11	ALL	A1 +	2	4	6	1	1	2	2	2	2. ALL	1	4.8	1	6.1	3	14x10^3	3	29x10^3
																	2	5.2	1	7.8	4	29x10^3	4	36x10^3
																					5	36x10^3	5	42X10^3
3	PRASANTH	331/08	12	M	30	AML	A +	4	29	33	2	2	2	2	2	3. AML	1	5	1	6.8	5	4x10^3	2	9X10^3
																	2	6.8	1	7.2	6	8x10^3	2	19X10^3
																	3	7.1	1	8	7	19x10^3	4	32X10^3
																	4	7.2	1	8.2	8	32x10^3	3	17X10^3
																					9	17x10^3	1	20X10^3
																					10	20x10^3	3	28X10^3
																					11	28x10^3	1	31X10^3
																					12	31x10^3	1	36X10^3
																					13	36x10^3	1	39X10^3
																					14	39x10^3	2	48X10^3
																					15	48x10^3	2	59X10^3
																					16	59x10^3	1	63X10^3
																					17	63x10^3	1	65X10^3
																					18	65x10^3	2	71X10^3
																					19	71x10^3	3	84x10^3
4	SURYA	1737/07	2	M	12	AML	O +	-	2	2	1	1	2	1	2	4.AML					1	40x10^3	2	19X10^3
5	PRIYADARSHINI	359/08	3.5	F	13	ALL	B +	4	5	9	2	2	2	2	2	5.ALL	1	5.2	1	7.1	1	40x10^3	2	57X10^3
																	2	7.1	1	7.9	2	57x10^3	2	67X10^3
																	3	6.1	1	7.2	3	67x10^3	1	75X10^3
																	4	7.2	1	8.8				
6	NIROSHA	514/08	10	F	25	ALL	B +	1	3	4	2	2	1	2	2	6. ALL	1	5.2	1	7.1	1	26x10^3	3	56X10^3
7	SURYA	1026/08	11	M	28	ALL	B +	1	-	1	1	1	2	1	2	7.ALL	1	6.8	1	7.4				
8	SHARUKA	1552/07	7	M	22	ALL	B +	2	2	4	2	2	2	1	2	8. ALL	1	7	1	8.2	1	120x10^3	1	125X10^3
																	2	7.8	1	8	2	80x10^3	1	85X10^3
9	JOSHUA	846/03	11	M	28	ALL	O +	-	3	3	1	1	2	1	2	9. ALL					1	10x10^3	3	25X10^3
10	VIJAY	220/08	5	M	18	ALL	O +	1	2	3	1	2	2	2	2	10. ALL	1	6.8	1	7.2	1	10x10^3	2	18X10^3
11	LATHA	1623/07	2	F	12	ALL	B +	1	1	2	1	1	2	2	2	11. ALL	1	5.2	1	6.4	1	11x10^3	1	15X10^3
12	KARTHIKA	711/08	12	F	31	ALL	B +	1	-	1	2	2	2	2	2	12. ALL	1	6.2	1	7.8				
13	VENKATESH	273/08	3.5	M	12	ALL	A1 +	4	7	11	1	1	1	2	2	13. ALL	1	7.2	1	8	1	9x10^3	3	20X10^3
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																	4	8.9	1	10.1	4	10x10^3	1	16X10^3
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																	2	10.8	1	11.9	2	7.2x10^3	1	13.3X10^3
																	3	4.9	1	5.4	3	13.3x10^3	1	19.8X10^3
																	4	5.4	1	7	4	19.8x10^3	1	22.4X10^3
																					5	22.4x10^3	2	31.3X10^3
																					6	31.3x10^3	2	39X10^3
																					7	39x10^3	4	52X10^3
																					8	52x10^3	1	59.3X10^3
																					9	59.3x10^3	1	62X10^3
																					10	62x10^3	1	68.7X10^3
																					11	68.7x10^3	3	82.4X10^3
																					12	70x10^3	1	74X10^3
																					13	72x10^3	1	76X10^3
15	PRADEEP	873/08	4	M	15	AML	O +	1	6	7	1	1	1	2	2	15. AML	1	2.1	1	3.4	1	75x10^3	2	87X10^3
																					2	87x10^3	2	93X10^3
																					3	93x10^3	2	100X10^3
16	ELUMALAI	609131	6	M	18	AML	B +	3	7	10	1	2	1	1	2	16. AML	1	7.8	1	9.2	1	5x10^3	1	8X10^3
																	2	9	1	9.8	2	8x10^3	1	10X10^3
																	3	9.8	1	10.5	3	10x10^3	1	16X10^3
																					4	16x10^3	1	20X10^3
																					5	20x10^3	2	32X10^3
																					6	32x10^3	1	38X10^3
																					7	38x10^3	1	46x10^3
17	RAHUL	605432	4	M	16	AML	B +	-	2	2	2	2	1	2	2	17. AML					1	90x10^3	1	100X10^3
18	MONICA	627/08	12	F	30	ALL	O +	-	1	1	1	2	1	2	2	18. ALL					1	37x10^3	1	41X10^3
19	SAI SIDDARTH	1540/07	9	M	22	AML	O +	1	28	29	2	2	1	1	2	19. AML	1	7	1	7.8	1	10x10^3	2	18X10^3
																					2	18x10^3	2	26X10^3
																					3	26x10^3	2	31X10^3
																					4	31x10^3	2	39X10^3
																					5	39x10^3	2	47X10^3
																					6	10x10^3	2	20X10^3
																					7	20x10^3	1	24X10^3
																					8	24x10^3	4	37X10^3
																					9	37x10^3	2	44X10^3
																					10	44x10^3	2	54X10^3
																					11	54x10^3	2	61X10^3
																					12	61x10^3	1	65X10^3
																					13	65x10^3	2	76X10^3
																					14	76x10^3	1	81X10^3
																					15	81x10^3	1	86X10^3
20	NANDA KUMAR	764/08	4.5	M	16	ALL	O +	2	6	8	1	2	1	2	2	20. ALL	1	7.6	1	8.8	1	10x10^3	2	18X10^3
																	2	8	1	9.2	2	18x10^3	2	29X10^3
																					3	10x10^3	1	16X10^3
																					4	16x10^3	1	24X10^3
21	FARHUL RAHUMAN	733/08	3.5	M	15	ALL	A1 +	1	8	9	1	1	2	2	2	21. ALL	1	3	1	4.1	1	4x10^3	1	11X10^3
																					2	11x10^3	2	18X10^3
																					3	15x10^3	2	24X10^3
																					4	25x10^3	2	31X10^3
																					5	30x10^3	1	36X10^3

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